STRUCTURE RELATED MEMBRANE POLARIZATIONS IN FIELD STIMULATED GUINEA PIG PAPILLARY MUSCLE

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Abstract- **We have performed optical potential mapping with very high temporal and spatial resolution in guinea pig papillary muscles during the application of electrical pulses. We used a surface staining procedure, with the potential sensitive dye di-4-ANEPPS, throughout our experiments which limited the depth of contribution to the optical signal to less than 40 µm [1]. Our results gave clear evidence, that the structural inhomogeneities themselves, in regions within about a space constant (< 1 mm), were able, during the application of an electrical pulse, to cause strong membrane polarizations. These polarizations, occurring far from electrodes, were sufficient to trigger local electrical activities. Our findings strongly support hypotheses, which assume, that the tissue structure – especially in regions far from electrodes or from the surface of the heart - play an important role in the defibrillation process.**

Keywords **- optical potential mapping, field stimulation**

I. INTRODUCTION

Field induced transmembrane potential changes are the necessary link to the resulting electrical interferences such as e.g. cardiac stimulation or defibrillation work in cardiac tissue. As predicted in several mathematical models, and measured in biological preparations, the tissue structure with its specific shape, dimension and inhomogeneities play a major role in the generation of these pulse induced membrane voltages. It is still an open question, to which extent inhomogeneities of various size scales can contribute to these polarization induced membrane voltages, which in turn may elicit an action potential, or even stop fibrillation. As was shown in an earlier study by Müller et al. [2] small inhomogeneities in guinea pig papillary muscles caused severe distortions in the spread of excitation. In the present paper we used the same type of preparation to study field induced membrane voltages, appearing within the range of 1 mm (i.e. within about a space constant).

II. METHODOLOGY

Guinea pig papillary muscles were stained with the voltage sensitive dye di-4-ANEPPS and mounted in a tissue bath. During the whole experiment the preparation was kept under near physiological conditions (Tyrode's solution at 36 ^{o} C, Oxygen saturated). A pair of current driven platinum electrodes was used to deliver electrical pulses with the electrical field strength oriented parallel to the main axis of the muscle. During a measurement, the green light of a frequency doubled Neodymium-Yag laser (100 mW, 532 nm) was used to excite the fluorescence of the stained specimen, which showed a voltage sensitivity of up to 10 % intensity change per 100 mV membrane potential change. In most cases, a few milliseconds before and after a measurement, the specimen was excited for 2 ms with the violet light of an Argon-Ion laser (457 nm). At this wavelength the voltage sensitivity of the dye is small compared to that of a green-light excitation [3]. An evaluation of the intensity of the fluorescence light before, during and after a measurement allowed us to compensate for baseline drifts of the optical signal caused by dye bleaching. The fluorescence light was projected onto an array of 8 x 8 photodiodes. The measured area was 1 x 1 mm; the distance between measuring spots (center to center) was 130 µm. Data acquisition was performed with a multichannel AD-converter (National Instruments). In this study electrical pulses (5 ms) in the range of 2 to 11 V/cm were applied during the resting state of the specimen. The optical signals thus obtained were low pass filtered and calibrated to typical action potential values (resting potential –84 mV, action potential amplitude 128 mV). A characteristic measure of the polarization induced membrane voltages (i.e. a polarization map) was gained by picking up the values of the calibrated optical signals at 200 µs after pulse onset. Within this short duration no noticeable electrical activity would disturb the (passive) field induced potential distribution. Activation maps were obtained by calculating the occurrence times of the maximum upstroke velocities of the elicited action potentials.

III. RESULTS

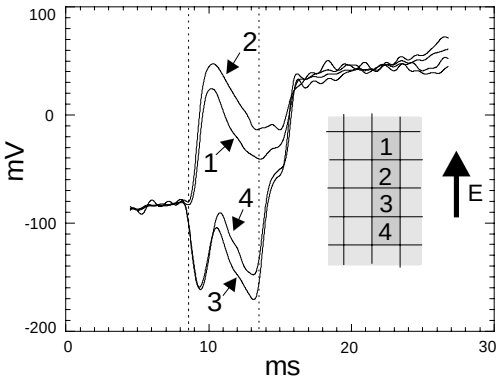

Fig. 1. Field stimulation in guinea pig papillary muscle. Overlay plot of calibrated optical signals. In the inset 4 consecutive measuring spots (distance center to center 130 µm) and the direction of the applied field (11 V/cm, 5 ms-pulse) are indicated. The related signals 1 to 4 indicate a pronounced inhomogenity in the region within spot 2 and 3.

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Abstract

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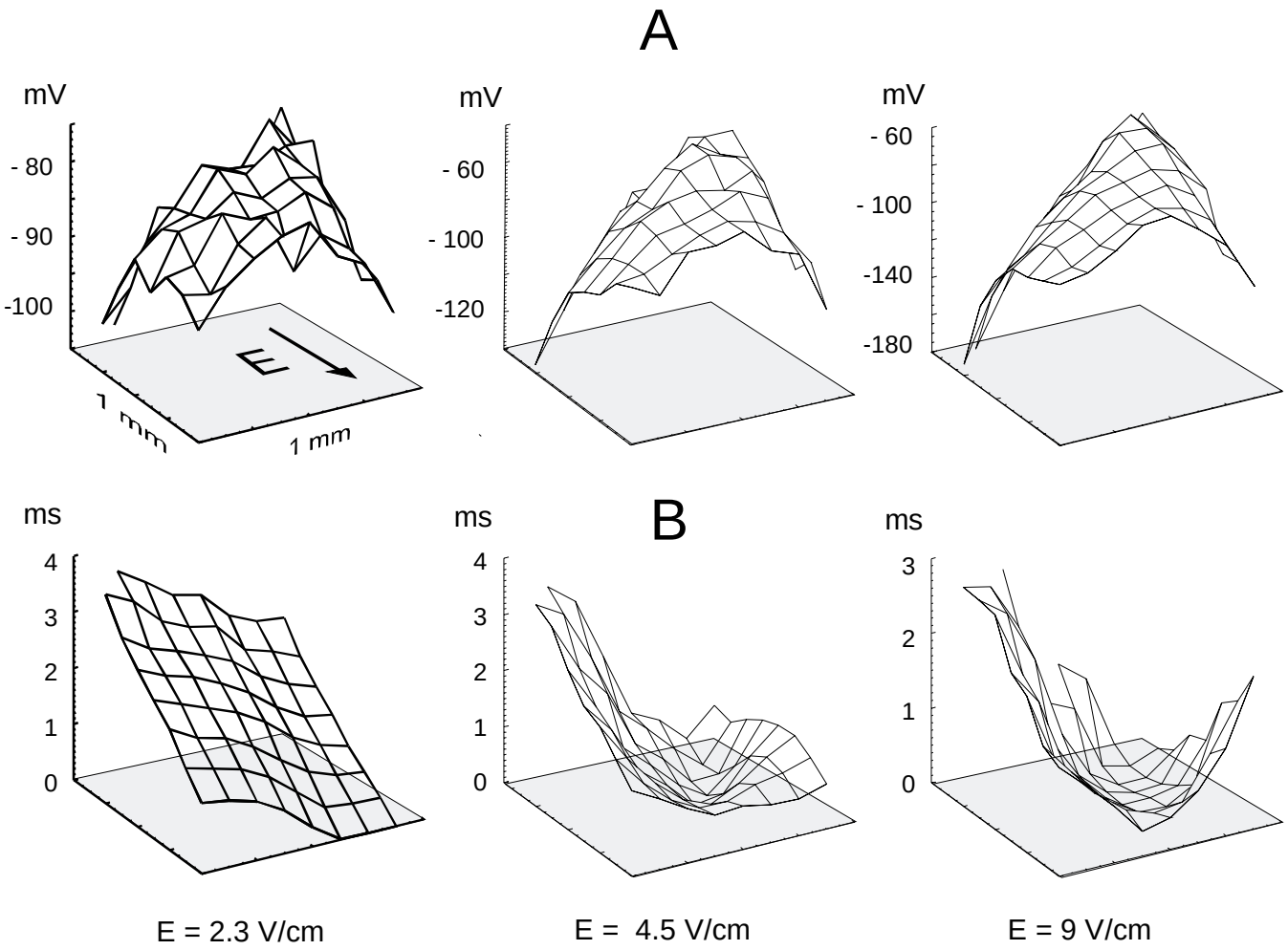

Fig. 2. Field stimulation in guinea pig papillary muscle. A: polarization maps, obtained from the calibrated optical signals 200 µs after pulse onset. The measuring area was 1 x 1 mm², the direction of the electrical field (pulse, 5 ms) is indicated. B: the corresponding activation maps, each map beginning with a zero-delay for the first occurence of the maximum upstroke velocity within the measuring area. The polarization maps (A) indicate an inhomogeneous region. With increasing field strenght, their shapes remain essentially constant. In contrast, the activation maps at the low field stregth (2.3 V/cm) indicate a relatively undisturbed travelling wave, but changes dramatically at higher field strength (4.5 V/cm and 9 V/cm). The activation now started in the region with the highest polarization (A)

In Fig. 1 and 2 some results from two experiments are presented. In both experiments the influence of the tissue structure on the local membrane polarizations and on the elicited electrical activities are clearly visible. In Fig. 1 an overlay plot of 4 calibrated optical signals, obtained simultaneously from 4 consecutive measuring spots within a colon of the array, is shown .

The labelled spots and the direction of the electrical field are indicated in the inset. The dotted lines indicate the onset and offset of the electrical pulse, which had an amplitude of 11 V/cm. Shortly after the onset of the electrical field signals 1 and 2 show nearly the same depolarisation, whereas signals 3 and 4 show a nearly identical hyperpolarization. In a homogeneous tissue, moving in equidistant steps from one site to another, would always lead to a smooth change of the local polarizations. In our experiment the sudden change of the membrane polarization between site 2 and 3 (from a pronounced depolarisation to a pronounced hyperpolarization), clearly indicate a inhomogeneous region, appearing within the distance of 130 µm (the distance between measuring spots). Despite the roughly equal Depolarization of the signals 1 and 2, and the equal hyperpolarization of the signals 3 and 4, the corresponding amplitudes during the pulse were quite different due to the different polarizations and electrical activities in the neighbourhood.

In Fig. 2 in the upper row (A) three polarization maps with increasing field strength are shown. The maps, taken 200 µs after pulse onset, show throughout the same shape. Despite a homogeneous electrical field was applied (the direction is indicated by the arrow), the local polarization distribution

was rather inhomogeneous. In the lower row (B) the corresponding activation maps are shown. At the low field strength of 2.3 V/cm the map indicates a smooth propagation which entered the measuring area next to the lower right corner. At 4.5 V/cm the map has changed dramatically: The activation now started within the measuring area in the region with the most prominent polarization (upper row). At 9 V/cm, compared to the map of 4.5 V/cm, the shape of the activation map remained nearly the same.

IV. Discussion

A. Methodological Aspects

Optical membrane potential mapping allows the monitoring of membrane potential changes even during the application of strong electrical pulses. We have used this method to gain a better insight in field induced electrical occurrences in cardiac tissue within the range of about a space constant (< 1 mm). The application of the electrical pulses during the resting state of the papillary muscle permitted both, a measure of the local polarizations within the first 200 µs (i.e. practically before the onset of the fast inward current) and, by evaluating the occurrence times of the maximum upstroke velocities, an activation map. In addition, in some experiments the pulses were applied during the plateau-phase of an action potential. The spatial resolution of a measurement is determined by both, the size of a measuring spot and its distance to the adjacent ones, as well as the depth in the specimen, in which fluorescence light is emitted and contributes thus to the optical signal. In our experiments we used surface staining throughout [4], which limited the depth of fluorescence to about 40 µm [1].

B. Field induced Membrane Voltages

The results shown clearly demonstrate the influence of the tissue structure on the local field-induced membrane polarizations. The results in Fig. 2 not only show a very inhomogeneous membrane polarization within the measuring field (row A), but gives evidence, that the induced polarization can determine the activation pattern of the tissue (row B). As indicated by the activation maps, the weak electrical pulse (field strength 2.3 V/cm) was able to induce an activation wave somewhere in the tissue near the cathode, but was not sufficient to cause a significant disturbance of the travelling wave within the measuring field. In contrast, the higher pulse amplitudes (field strength 4.5 and 9 V/cm) caused the wave to start within the measuring field in the region of maximum depolarization. The tissue in this area was not depolarized due to the neighbourhood to the cathode (which would raise the membrane potentials within the whole area), but only due to the local inhomogeneities (the mean polarization in that area was slightly negative, which never would trigger any activation during the pulse).

V. Conclusion

Our experiments clearly demonstrate, that an inhomogeneous region in the range of a space constant during a strong electrical pulse can lead to membrane polarizations, which are strong enough to trigger a local electrical activity. Hypotheses, which assume that the tissue structure itself – especially in regions far from the electrodes or the surface of the heart - play an important role in a defibrillation process, are thus strongly supported. Our results also show, that prominent polarization changes can occur in distances of about a cell length (130 µm). Further investigations in various heart tissue preparations will be necessary to identify those inhomogeneities, which are the most important ones in a successful defibrillation treatment.

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